Detection (FISH)

Introduction

Probes labeled with biotin must be detected with fluorescently labeled Avidin, and probes labeled with digoxigenin require detection with a fluorochrome conjugated antibody against this hapten. For example, to detect the biotin labeled probes we routinely use Avidin-FITC, Avidin-TRITC, or Avidin Cy-5. For the probes labeled with digoxigenin, we usually first incubate with mouse-anti-digoxigenin, followed by incubation with sheep anti-mouse Cy5.5, or other fluorochrome conjugated antibodies.

Reagents

Avidin-Cy5
Jackson Immuno Research Lab Cat 003-170-083
Avidin-TRITC
Sigma Cat. A 7169
Avidin-FITC
Vector Cat. A-2011
BSA (Bovine Serum Albumin)
DAPI
Ethanol, absolute
Formamide
Fluka BioChemika Cat 47671
HCl 1N
Mouse anti-digoxigenin
Sigma, Cat. D 8156
Sheep anti-mouse Cy5.5
Amersham, Cat. RPQ 0115
SSC 20X
Tween 20

Preparation of Reagents

50% FA/SSC

SSC 20X	30 ml
dH_2O	120 ml
Formamide	150 ml

Adjust pH to 7with 1N HCl
Pre-warm to 45°C

1X SSC (for direct labeled probes, i.e., TRITC, FITC or other)

SSC 20X	25 ml

dH_2O 475 ml
Pre-warm to 45°C

0.1X SSC (for indirect labeled probes, i.e. Biotin, or Digoxigenin)
SSC 20X 2.5 ml
dH2O 497.5 ml
Pre-warm to 60°C

4X SSC/0.1%Tween20
SSC 20X 200 ml
dH_2O 799 ml
Tween 20 1 ml
Pre-warm to 45°C

Blocking Solution (3% BSA/4X SSC/0.1%Tween20)
BSA 0.3 g
4X SSC/0.1%Tween 20 10 ml
Pre-warm to 37°C

Antibody Solution (1% BSA/4X SSC/0.1%Tween 20)
BSA 0.1 g
4X SSC/0.1%Tween 20 10 ml
Pre-warm to 37°C

DAPI stock solution (f.c.= 0.2mg/ml)
DAPI 2 mg
ddH_2O 10 ml
Aliquot and store at -80ºC

DAPI staining solution (f.c.= 80ng/ml)
DAPI (stock solution) 40 µl
SSC 2X 100 ml
Store at 4˚C in a light-tight coplin jar

Procedure

1. Carefully remove the rubber cement surrounding the coverslips from hybridized slides.

2. Wash the slides in 50% formamide/2xSSC (pH 7-7.5) for 3 x 5 min, shaking.

3. Wash slides in 0.1X SSC (for indirectly labeled probes) or 1X SSC (for directly labeled probes) for 3 x 5 min, shaking.

4. Dip slides in 4X SSC/0.1%Tween 20.

5. Add 120 µl of Blocking Solution (3% BSA/4X SSC/0.1%Tween 20) to the slides and over them with a 24 mm x 60 mm coverslip in a moist hybridization chamber at 37°C for 30 min.

6. Dip slides in 4X SSC/0.15Tween 20 to wash off the blocking solution. Proceed directly to step 9 if using a directly-labeled probe.

7. For indirectly-labeled probes (Biotin or Digoxigenin), add 120 µl of fluorescent antibody (antibody should be diluted 1:200 in 1% BSA/4X SSC/0.1%Tween 20) to the slides, cover with a 24 mm x 60 mm coverslip, and incubate in moist light-tight hybridization chamber at 37°C for 45 min.

8. Wash slides in 4X SSC/0.1%Tween 20, for 3 x 5 min, shaking.

9. Stain slides for 5 min in DAPI staining solution in a light-protected coplin jar.

10. Wash the slides for 5 min in 2X SSC, shaking.

11. Dehydrate the slides by dipping through 2X SSC followed by washes with an increasing ethanol series of 70%, 90%, and100%; air-dry.

12. Apply 35 µl of antifade solution, cover with 24 mm x 60 mm coverslips, store in light-protected container at 4ºC until slide is imaged.

Notes

1. Exposure of slides to ambient light should be minimized during all procedures.

2. Use care in removing coverslips during all procedures to minimize scratches.

3. Spin all fluorescent dyes prior to use for 3 min at 13,000 rpm and carefully pipette the antibody without disturbing the pellet..

4. Do not let the slide dry out between washing steps.